

EXHIBIT 97

ORIGINAL ARTICLE

Decreased Catalytic Activity and Expression of Protein Kinase C Isozymes in Teenage Suicide Victims

A Postmortem Brain Study

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Background: Teenage suicide is a major public health concern. Although there is some understanding of the psychosocial factors associated with teenage suicide, little is known about the neurobiologic factors of teenage suicide. Protein kinase C (PKC) is a critical phosphorylating enzyme in the phosphoinositide signaling pathway (which is involved in many physiologic functions in the brain and has been implicated in the pathogenesis of mood disorders) and is also a target for the therapeutic action of mood-stabilizing drugs.

Objective: To examine whether the pathogenesis of teenage suicide is associated with changes in PKC.

Design: Postmortem brain study.

Participants: Seventeen teenage suicide victims and 17 nonpsychiatric control subjects.

Main Outcome Measures: Catalytic activity of PKC and protein and messenger RNA levels of various PKC isozymes, such as PKC α , β , and γ , were deter-

mined in the prefrontal cortex and hippocampus of both groups.

Results: Protein kinase C activity was statistically significantly decreased in membrane and cytosol fractions of the prefrontal cortex and hippocampus of teenage suicide victims compared with control subjects. Statistically significant decreases in protein levels of PKC α , β I, β II, and γ isozymes were also observed in both of these fractions. These decreases were associated with decreases in levels of their respective messenger RNAs.

Conclusion: Because many physiologic functions are mediated through phosphorylation by PKC and because PKC is a target for the therapeutic action of psychoactive drugs, our findings indicate that the pathogenesis of teenage suicide may be associated with abnormalities in PKC and that PKC may be a target for therapeutic intervention in patients with suicidal behavior.

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SUICIDE IS A MAJOR PUBLIC health concern,¹ and approximately 30 000 people die of suicide each year in the United States alone.² Teenage suicide is a special concern because it is the second leading cause of death among teenagers and because the suicide rate in the past 10 to 20 years has increased 4-fold in the white teenage male population.³ Whereas there is some understanding of the psychosocial and psychological factors associated with teenage suicide and of the neurobiologic abnormalities of adult suicide, little is known about the neurobiologic characteristics of teenage suicide. Studies⁴⁻⁹ of the neurobiologic factors in adult suicide have indicated abnormalities in neurotransmitter receptors, such as 5-hydroxytryptamine 2A (5-HT_{2A}) and α - or β -adrenergic receptors, and in the receptor-linked signaling

systems, namely, adenylate cyclase and the phosphoinositide signaling system, to which these receptors are linked.

The characteristics of teenage suicide may be similar to those of adult suicide in some respects but may differ in others. Besides psychiatric disorders, other risk factors for suicide include psychosocial stressors, impulsivity, and aggression.¹⁰ In teenagers, the major driving factor leading to suicide is aggressive/impulsive behavior,^{10,11} which has been implicated in abnormalities in serotonin function.¹² In a recent study, our group⁵ found that the number of 5-HT_{2A} receptors was increased in the prefrontal cortex (PFC) and hippocampus of suicide victims, which was associated with an increase in protein and messenger RNA (mRNA) expression. Although these studies indicated abnormalities in 5-HT_{2A} receptors, the biochemical

consequences and the physiologic significance of this observation remain unclear.

Protein kinase C (PKC) is an important component of the phosphoinositide signaling system^{13,14} to which 5-HT_{2A} and several other receptors, such as 5-HT_{2C}, α_1 -adrenergic, and muscarinic M₁ receptors, are linked and mediate their functional response. In the phosphoinositide signaling system, stimulation of these receptors activates the effector phospholipase C, which causes hydrolysis of the substrate inositol-4,5-bisphosphate and results in the formation of 2 second messengers, diacylglycerol and inositol triphosphate.¹⁵ Diacylglycerol activates the phospholipid- and calcium-dependent PKC and increases the affinity of the enzyme for calcium.¹⁶ Inositol triphosphate, on the other hand, mobilizes calcium from intracellular stores.¹⁴ Activation of PKC is associated with translocation of the enzyme from cytoplasm to membrane.¹⁷ Once PKC has been activated, it is involved in the phosphorylation of several membranous, cytosolic, and nuclear proteins.

Protein kinase C is a key regulatory enzyme present in various tissues, including the brain, and it is localized presynaptically and postsynaptically.^{13,18} The expression of PKC in the brain region is isoform specific.¹⁹ Heterogeneity exists in PKC, and 12 different isozymes have now been described.¹⁶ Each individual isozyme is involved in specific cellular responses, such as migration, proliferation, atrophy, apoptosis, and secretion, which suggests that these isozymes are important in clinical disorders.^{14,19-21} The PKC family has been subgrouped into 3 classes: conventional, which includes α , β I, β II, and γ ; novel, which includes δ , ϵ , and ζ ; and atypical.^{14,16} Each isozyme is encoded by a unique gene, except for β I and β II isozymes, which are the splicing products of the same transcript.²² The biochemical properties of each isozyme have been identified with respect to activation or to phosphorylation, proteolytic activation, degradation, and substrate specificity.²²

There is direct and indirect evidence^{20,23,24} suggesting that PKC may play a crucial role in mental disorders. An earlier study²⁵ examined the role of PKC in suicide by determining [³H]phorbol 12,13-dibutyrate (PDBu) binding in the PFC and hippocampus of teenage suicide victims. [³H]phorbol 12,13-dibutyrate is a highly specific ligand that measures regulatory subunits of PKC.^{26,27} The authors²⁵ found that [³H]PDBu binding was significantly decreased in the PFC and hippocampus of teenage suicide victims compared with nonpsychiatric control subjects. This study provided preliminary evidence of abnormalities of PKC in teenage suicide; however, [³H]PDBu, as stated previously, measures only regulatory subunits of PKC, and it was not clear from this study whether the decrease in PKC binding sites was associated with either changes in catalytic activity or changes in the level of any specific isozyme. Because each isozyme is related to specific functions and is region specific, we determined the catalytic activity of PKC and the protein levels of various isozymes in membrane and cytosol fractions of the PFC and hippocampus obtained from postmortem brain samples of teenage suicide victims and nonpsychiatric control subjects. To further examine whether any changes in the isozymes are related to altered transcription, we determined the mRNA levels of these isozymes in total RNA.

METHODS

PARTICIPANTS

The right hemispheres of the PFC (Brodmann area 9) and hippocampus from 17 teenage suicide victims and 17 nonpsychiatric teenage control subjects obtained from the Brain Collection Program of the Maryland Psychiatric Research Center, in collaboration with the Medical Examiner's Office of the State of Maryland, were used. Brain samples were free of neuropathologic abnormalities and human immunodeficiency virus antibodies. Toxicologic data were obtained by analysis of urine and blood samples from the participants.

All patients in this study were diagnosed based on the *Diagnostic Evaluation After Death*²⁸ and the *Structured Clinical Interviews for the DSM-III-R* as described earlier.²⁹ Two senior psychiatrists provided independent lifetime DSM-III-R diagnoses. Controls were verified as being free of mental illness and substance abuse. The protocols for tissue sampling and retrospective assessments were approved by the institutional review board of the University of Maryland. This study was also approved by the institutional review board of the University of Illinois at Chicago. The demographic, clinical, and toxicologic characteristics of the participants are provided in the **Table**.

DETERMINATION OF PKC ACTIVITY IN MEMBRANE AND CYTOSOL FRACTIONS

Protein kinase C activity in membrane and cytosol fractions of both brain areas was measured by using the procedure described in a previous study.³⁰ Assay tubes contained 25 μ L of a component mixture (3mM Ca/[C₃H₅O₂]₂, L- α -phosphatidyl-L-serine [75 μ g/mL], phorbol 12-myristate 13-acetate [6 μ g/mL], 225 μ M substrate peptide, and 7.5mM dithiothreitol in 50mM Tris hydrochloride containing 0.05% sodium azide, pH 7.5) and 25 μ L of the membrane or cytosol fraction. The reaction was initiated by the addition of 25 μ L of magnesium-adenosine triphosphate buffer ([γ -³²P]adenosine triphosphate [10 μ Ci/mL {370000 Bq/mL}], 1.2mM adenosine triphosphate, 72mM magnesium chloride, and 30mM HEPES [4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid], pH 7.4) and incubated for 15 minutes at 37°C. The reaction was terminated by the addition of 100 μ L of 300mM orthophosphoric acid. An aliquot of the solution from each tube (35 μ L) was blotted onto individual peptide-binding papers, and the retained radioactivity was counted.

QUANTITATION OF PKC ISOZYMES IN MEMBRANE AND CYTOSOL FRACTIONS BY WESTERN BLOT

Immunolabeling of PKC α , β I, β II, and γ was determined as described in a previous study.³⁰ Equal volumes of tissue samples (30 μ g of protein in 20 μ L) were loaded onto 7.5% (weight per volume) polyacrylamide gel and electrophoresed. The blots were initially developed using polyclonal anti-PKC α , β I, β II, or γ antibody (1:3000-1:5000 dilution) and subsequently using β -actin monoclonal antibody (1:5000 dilution). The levels of PKC isozymes were calculated as a ratio of the optical density of the PKC antibody of interest to the optical density of β -actin antibody.

QUANTITATIVE RT-PCR ANALYSIS OF mRNA LEVELS OF PKC ISOZYMES AND NEURON-SPECIFIC ENOLASE

Messenger RNA levels of PKC isozymes and neuron-specific enolase (NSE) were determined in total RNA as described in detail in a previous study.³¹ Internal standards were used to determine the quantitation of PKC isozyme and NSE mRNAs.³¹

Demographic, Clinical, and Toxicologic Characteristics of 17 Teenage Suicide Victims and 17 Control Subjects

Participant No.	Age, y*	Sex	Race	PMI, h†	pH‡	Diagnosis	Toxicologic Evidence	Cause of Death
Control Group								
1	19	M	Black	6	6.14	Control	None	GSW
2	13	M	White	Not available	5.19	Control	None	Accident
3	16	M	Black	6	6.54	Control	None	GSW
4	19	M	Black	9	6.85	Control	None	GSW
5	16	M	Black	8	5.64	Control	None	GSW
6	18	M	White	8	6.7	Control	None	Stabbing
7	19	M	Black	12	5.9	Control	None	GSW
8	17	M	Black	11	6.52	Control	None	GSW
9	16	M	White	10	5.42	Control	None	Stabbing
10	18	M	Black	11	6.16	Control	None	GSW
11	17	M	Black	10	5.9	Control	None	GSW
12	13	M	Black	22	6.07	Control	None	GSW
13	14	M	Black	18	5.73	Control	None	GSW
14	18	M	Black	27	6.09	Control	None	Drowning
15	16	M	White	21	5.97	Control	None	Hanging
16	17	F	Black	26	6.23	Control	None	Multiple injuries
17	18	M	White	19	6	Control	None	Drowning
Suicide Victims								
18	20	F	White	11	6.5	Schizophrenia	None	Jump from height
19	19	M	White	12	5.9	Drug abuse	None	Hanging
20	15	F	White	7	5.48	Alcohol abuse	Alcohol	GSW
21	12	M	Black	10	5.91	Major depression	None	Hanging
22	15	F	White	11	6.44	No mental disorder	Phenylpropanolamine, chlorophenylamine, codeine, salicylate, meglumine, acetaminophen	Drug overdose
23	17	M	White	11	5.9	No mental disorder	None	GSW
24	16	M	Black	12	5.4	No mental disorder	None	Drug overdose
25	13	M	White	18	6	Hyperactive and attention deficit	Methylphenidate hydrochloride	Hanging
26	13	M	Black	11	5.37	No mental disorder	None	Hanging
27	15	M	White	11	5.33	Major depression	None	Asphyxia
28	15	F	White	17	5.58	Major depression and hyperactive and attention deficit	Imipramine hydrochloride and desipramine hydrochloride	Drug overdose
29	16	M	Other	20	6.17	No mental disorder	None	Hanging
30	17	F	White	25	5.55	Adjustment disorder	Verapamil	Drug overdose
31	15	M	White	27	6.08	Adjustment disorder and major depression	Pseudoephedrine, phenylpropanolamine	GSW
32	16	F	White	18	6.31	No mental disorder	Amitriptyline hydrochloride	GSW
33	16	F	White	33	6.61	Adjustment disorder	None	GSW
34	16	M	White	24	6.81	Adjustment and conduct disorder	None	Hanging

Abbreviations: GSW, gunshot wound; NA, not applicable; PMI, postmortem interval.

*Mean \pm SD age was 16.71 \pm 1.93 years in controls and 15.65 \pm 1.99 years in suicide victims.

†Mean \pm SD PMI was 13.97 \pm 7.1 in controls and 16.12 \pm 7.54 in suicide victims.

‡Mean \pm SD pH was 6.04 \pm 0.41 in controls and 5.96 \pm 0.47 in suicide victims.

Primer pairs were designed to allow amplification of 700 to 1003 base pairs (bp) for PKC α (GenBank accession number X52479), 914 to 1399 bp for PKC β (GenBank accession number X06318), 1506 to 1823 bp for PKC γ (GenBank accession number AF345987), and 295 to 675 bp for NSE (GenBank accession number X14327). The internal primers for PKC isozymes and NSE were as follows: PKC α , 843 to 866 bp; PKC β , 1131 to 1155 bp; PKC γ , 1645 to 1668 bp; and NSE, 403 to 423 bp. Internal standards contained *Bgl*III (PKC isozymes) or *Xho*I (NSE) restriction endonuclease sites. Decreasing concentrations of PKC isozyme or NSE internal standard complementary RNAs were added to 1 μ g of total RNA. The polymerase chain reaction mixture was amplified for 30 cycles, digested with *Bgl*III or with *Xho*I in triplicate, and run on 1.5% agarose

gel. The results were calculated as the counts incorporated into the amplified complementary RNA standard divided by the counts incorporated into the corresponding mRNA amplification product vs the known amount of PKC isozyme or NSE internal standard added to the test sample. The results are expressed as attomoles per microgram of total RNA.

STATISTICAL ANALYSIS

Data analyses were performed using a statistical software package (SPSS 8.0; SPSS Inc, Chicago, Ill). All values are reported as mean \pm SD. Differences in PKC catalytic activity, mRNA and protein levels of PKC isozymes, age, sex, postmortem interval (PMI), and pH of the brain between suicide victims and con-

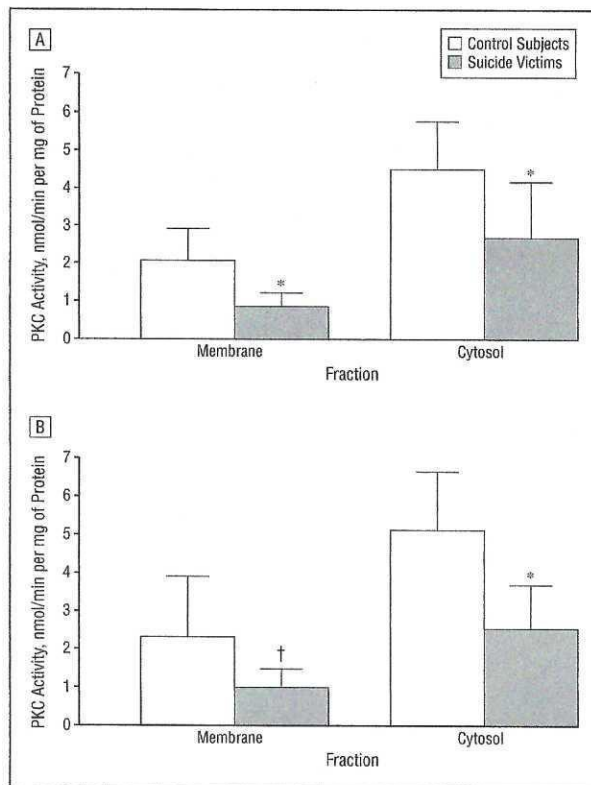


Figure 1. Mean protein kinase C (PKC) catalytic activity in membrane and cytosol fractions of the prefrontal cortex (A) and hippocampus (B) obtained from 17 control subjects and 17 teenage suicide victims. Error bars represent SD. Asterisk indicates $P < .001$; dagger, $P = .002$.

control subjects were analyzed using the independent-sample *t* test. The relationships between PKC catalytic activity, mRNA and protein levels of PKC isozymes, PMI, age, sex, and pH of the brain were determined by using Pearson product moment correlation analysis. *P* values were 2-tailed. Statistical differences between subgroups of suicide victims (with and without mental disorders) and controls were evaluated by using 1-way analysis of variance. During analysis of the data, we included race as a potential confounding variable.

RESULTS

The detailed demographic characteristics of the teenage suicide victims ($n = 17$) and the control subjects ($n = 17$) are given in the Table. There were no significant differences in age ($t_{32} = 1.5$; $P = .12$), PMI ($t_{31} = 0.84$; $P = .40$), or pH of the brain ($t_{32} = 0.51$; $P = .61$) between controls and suicide victims.

Protein kinase C activity was determined in membrane and cytosol fractions of the PFC and hippocampus (**Figure 1**). There was a significant decrease in PKC activity in membrane and cytosol fractions of the PFC and hippocampus of teenage suicide victims compared with control subjects (PFC: membrane, $t_{32} = 4.65$; $P < .001$; cytosol, $t_{32} = 3.59$; $P = .001$; and hippocampus: membrane, $t_{32} = 3.2$; $P = .003$; cytosol, $t_{32} = 2.77$; $P = .009$).

Figure 2 shows representative immunoblots of the various PKC isozymes in membrane and cytosol fractions obtained from the PFC of 2 teenage suicide victims and 2 control subjects. Protein kinase C α , β I, β II,

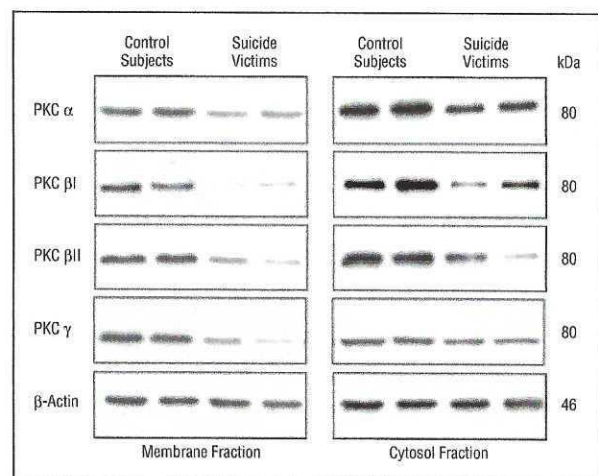


Figure 2. Representative Western blots showing the immunolabeling of protein kinase C (PKC) isozymes (PKC α , β , and γ) in membrane and cytosol fractions from the prefrontal cortex of 2 control subjects and 2 teenage victims. Protein samples were subjected to 10% polyacrylamide gel electrophoresis and transferred to chemiluminescence membranes, which were then incubated with primary antibodies specific for each PKC isozyme and secondary anti-rabbit antibody. The membranes were striped and probed with primary β -actin and secondary anti-mouse antibody. The bands were quantified as described in the "Methods" section. Ratios of the optical densities of PKC isozymes to that of β -actin were calculated.

and γ isozymes migrated to 80 kDa, whereas β -actin migrated to 46 kDa. Levels of PKC α , β I, β II, and γ decreased in membrane and cytosol fractions obtained from the PFC of teenage suicide victims. Mean levels of PKC α , β I, β II, and γ were significantly decreased in membrane and cytosol fractions of the PFC (**Figure 3**) and hippocampus (**Figure 4**) of teenage suicide victims compared with controls. The significance levels of these decreases in the PFC were as follows: membrane—PKC α , $t_{32} = 4.6$; $P < .001$; PKC β I, $t_{32} = 4.1$; $P < .001$; PKC β II, $t_{32} = 4.5$; $P < .001$; and PKC γ , $t_{32} = 3.3$; $P = .002$; cytosol—PKC α , $t_{32} = 3.5$; $P = .001$; PKC β I, $t_{32} = 4.1$; $P < .001$; PKC β II, $t_{32} = 3.8$; $P = .001$; and PKC γ , $t_{32} = 6.3$; $P < .001$. The significance levels of these decreases in the hippocampus were as follows: membrane—PKC α , $t_{32} = 3.2$; $P = .003$; PKC β I, $t_{32} = 3.8$; $P < .001$; PKC β II, $t_{32} = 2.6$; $P = .02$; and PKC γ , $t_{32} = 3.1$; $P = .004$; cytosol—PKC α , $t_{32} = 2.8$; $P = .009$; PKC β I, $t_{32} = 3.9$; $P < .001$; PKC β II, $t_{32} = 2.0$; $P = .04$; and PKC γ , $t_{32} = 2.4$; $P = .02$.

To examine whether decreases in PKC isozyme levels were related to altered transcription of their respective mRNAs, we determined the mRNA levels of the various PKC isozymes by using quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). Representative gel electrophoreses of the competitive RT-PCR of PKC isozymes are shown in **Figure 5A-C** for 1 control subject. The results of a competitive RT-PCR analysis for PKC α , β , and γ are shown in **Figure 5D-F**, where the point of equivalence represents the amount of PKC isozyme mRNAs present. The mRNA levels of PKC α , β , and γ were significantly decreased in the PFC (PKC α , $t_{30} = 5.5$; $P < .001$; PKC β , $t_{30} = 3.3$; $P = .002$; and PKC γ , $t_{30} = 3.7$; $P = .001$) and in the hippocampus (PKC α , $t_{30} = 5.5$; $P < .001$; PKC β , $t_{30} = 3.3$; $P = .002$; and PKC γ , $t_{30} = 3.7$; $P = .001$) of teenage suicide victims (**Figure 6**). To establish whether neuronal RNA contributes equally to the

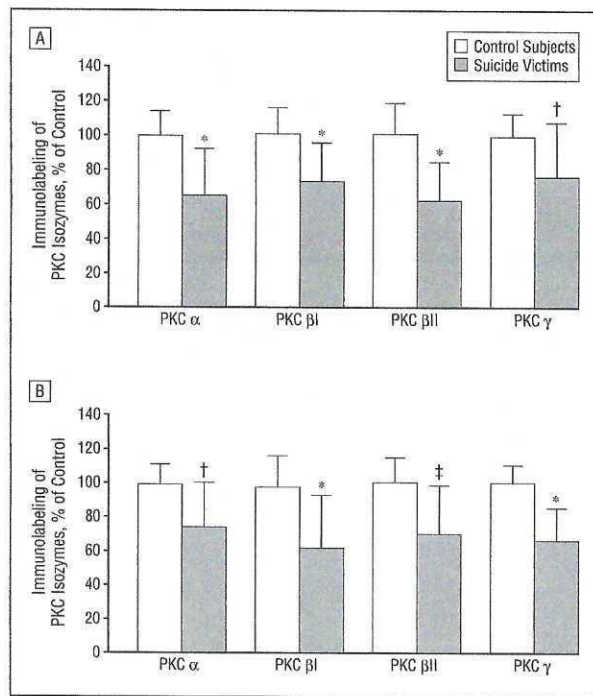


Figure 3. Mean protein levels of protein kinase C (PKC) α , β I, β II, and γ isozymes in membrane (A) and cytosol (B) fractions of the prefrontal cortex obtained from 17 teenage suicide victims and 17 control subjects. Error bars represent SD. Asterisk indicates $P=.001$; dagger, $P<.001$; and double dagger, $P=.002$.

total RNA pool, we determined the mRNA level of NSE in the PFC of suicide victims and control subjects. Levels of NSE mRNA in PFC in control subjects vs suicide victims showed no significant differences (360 ± 89 vs 408 ± 98 attomoles/ μ g of total RNA; $t_{30}=1.46$; $P=.15$). However, the ratios of PKC isozyme to NSE mRNA showed that PKC mRNA levels were significantly decreased in the PFC of suicide victims when expressed as a function of NSE mRNA (PKC α : control, 0.26 ± 0.11 ; suicide, 0.11 ± 0.04 ; PKC β : control, 0.46 ± 0.32 ; suicide, 0.15 ± 0.08 ; and PKC γ : control, 1.2 ± 0.22 ; suicide, 0.69 ± 0.20).

EFFECT OF CONFOUNDING VARIABLES AND DIAGNOSIS

We did not find any effect of age, PMI, and pH of the brain on any of the PKC measures in either the PFC or the hippocampus (data not shown). In addition, we examined whether antidepressant drug treatment affects any of the measures. Of 17 suicide victims, 3 had toxicologic evidence of antidepressant drugs. Comparison of suicide victims with toxicologic findings positive for antidepressant drugs with those without such findings revealed no significant differences in PKC activity or in protein and mRNA levels of PKC isozymes in the PFC and hippocampus between these 2 groups (data not shown).

Some previous studies²⁴ have shown an association between abnormalities in PKC activity and PKC isozymes in the platelets of patients with mood disorders. We, therefore, examined whether the decrease in PKC isozyme levels is associated with mental disorders or

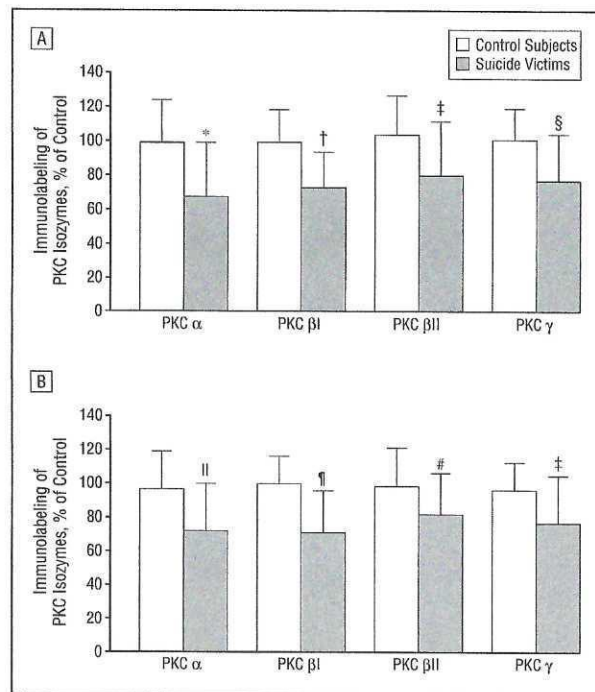


Figure 4. Mean protein levels of protein kinase C (PKC) α , β I, β II, and γ isozymes in membrane (A) and cytosol (B) fractions of the hippocampus obtained from 17 teenage suicide victims and 17 control subjects. Error bars represent SD. Asterisk indicates $P=.003$; dagger, $P=.001$; double dagger, $P=.02$; section mark, $P=.004$; parallel mark, $P=.009$; paragraph symbol, $P<.001$; and number sign, $P=.04$.

whether it is associated with suicide independent of diagnosis. Of the 17 suicide victims, 9 had a history of some kind of mental disorder, primarily mood disorders and adjustment disorders, and 8 had no history of mental disorders, although 2 had a history of alcohol or other drug abuse. When we compared the various PKC isozymes between patients who had a lifetime history of mental disorder and those who did not have such a history, there were no statistically significant differences between the 2 groups in catalytic activity of PKC or in protein expression of any of the PKC isozymes in membrane and cytosol fractions. Similarly, there were no significant differences in mRNA expression levels in patients who had a lifetime history of mental disorders and those who did not have such a history; however, both subgroups were significantly lower than control subjects in terms of protein and mRNA expression of the various PKC isozymes (data not shown). These results thus suggest that the decreases in protein and mRNA expression of PKC isozymes found in teenage suicide victims were independent of diagnosis.

EFFECT OF SEX ON PKC ACTIVITY AND ON PROTEIN AND mRNA LEVELS OF PKC ISOZYMES

There were 16 males and 1 female in the control group and 10 males and 7 females in the suicide group. To examine whether the uneven sex distribution had any effects on PKC, we analyzed the data in males in the control and suicide groups. We found significant differences in PKC activity (PFC: membrane, $t_{24}=3.08$; $P=.01$; cy-

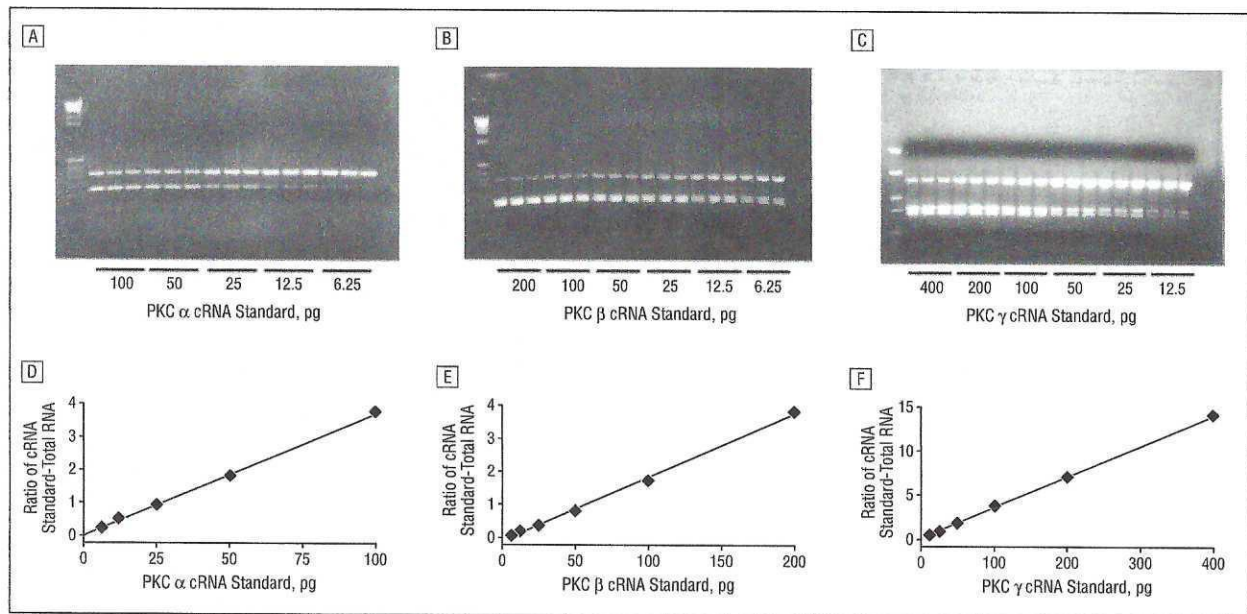


Figure 5. A representative experiment showing a competitive polymerase chain reaction analysis for protein kinase C (PKC) α (A), PKC β (B), and PKC γ (C) messenger RNA (mRNA) contents in the prefrontal cortex of 1 control subject. Decreasing concentrations of complementary RNA (cRNA) for PKC α (100-6.25 pg), PKC β (200-6.25 pg), or PKC γ (400-12.5 pg) were added to a constant amount (1 μ g) of total RNA. The mixtures were reverse transcribed and polymerase chain reaction amplified in the presence of trace amounts of [32 P]dCTP (cytidine triphosphate); aliquots were digested by *Xho* I and electrophoresed on 1.5% agarose gel. The higher molecular size band corresponds to amplification product arising from the mRNA, whereas the lower band arises from cRNA generated from the internal standard digested by *Xho* I. Data derived from the agarose gel are plotted as the counts incorporated into the amplified cRNA standard divided by the counts incorporated into the corresponding PKC α (D), PKC β (E), or PKC γ (F) mRNA amplification product vs the known amount of internal standard cRNA added to the test sample. The point of equivalence represents the amount of the respective mRNA.

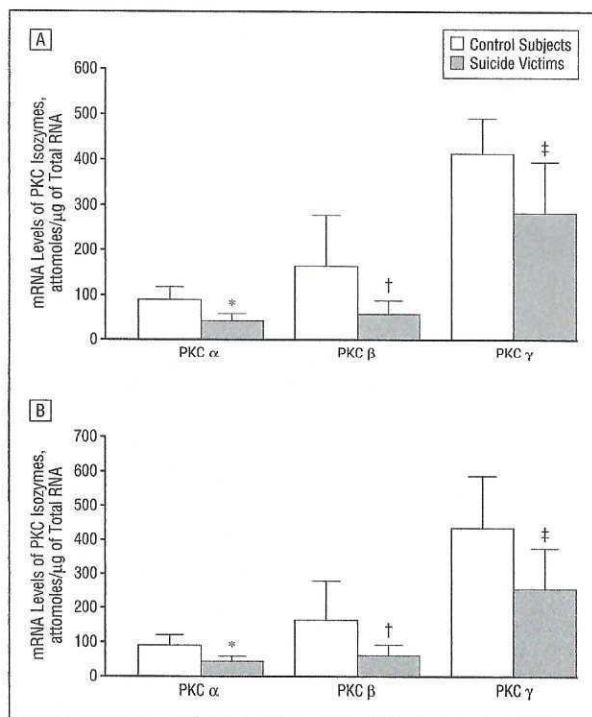


Figure 6. Mean messenger RNA (mRNA) levels of protein kinase C (PKC) α , β , and γ in the prefrontal cortex (A) and hippocampus (B) of 17 control subjects and 15 teenage suicide victims. Error bars represent SD. Asterisk indicates $P < .001$; dagger, $P = .002$; double dagger, $P = .001$.

tosol, $t_{24} = 4.2$, $P < .001$; and hippocampus: membrane, $t_{24} = 2.4$, $P = .02$; cytosol, $t_{24} = 4.04$, $P = .001$), protein levels (PFC—membrane: PKC α , $t_{24} = 3.9$, $P = .001$; PKC β ,

$t_{24} = 4.2$, $P < .001$; and PKC γ , $t_{24} = 2.7$, $P = .01$; and cytosol: PKC α , $t_{24} = 2.6$, $P = .01$; PKC β , $t_{24} = 4.7$, $P = .001$; and PKC γ , $t_{24} = 5.4$, $P < .001$; and hippocampus—membrane: PKC α , $t_{24} = 2.6$, $P = .01$; PKC β , $t_{24} = 3.4$, $P = .005$; and PKC γ , $t_{24} = 2.4$, $P = .02$; and cytosol: PKC α , $t_{24} = 2.0$, $P = .05$; PKC β , $t_{24} = 3.5$, $P = .002$; and PKC γ , $t_{24} = 2.2$, $P = .03$), and mRNA levels (PFC: PKC α , $t_{22} = 3.5$, $P = .002$; PKC β , $t_{22} = 2.4$, $P = .02$; and PKC γ , $t_{22} = 2.2$, $P = .03$; and hippocampus: PKC α , $t_{22} = 3.5$, $P = .002$; PKC β , $t_{22} = 2.4$, $P = .02$; and PKC γ , $t_{22} = 2.6$, $P = .01$) between controls and suicide victims with a similar magnitude as observed after inclusion of females. In the suicide group, we also analyzed the data comparing male and female suicide victims. We did not find any significant effects of sex on PKC activity or mRNA and protein levels of PKC isoforms (data not shown). These data suggest that the effects seen on PKC are not related to sex.

Because there was an uneven distribution of race between the control and suicide groups, we analyzed the effect of race by comparing PKC measures between blacks and whites in both groups and found no significant differences between them (data not shown). We also included race as a covariate in the analysis.

COMMENT

The results of the present study reveal a statistically significant reduction in the catalytic activity of PKC in the PFC and hippocampus of teenage suicide victims compared with control subjects. This reduction in PKC activity was associated with decreases in protein and mRNA expression of PKC α , β , and γ isoforms in the PFC and

hippocampus of teenage suicide victims. These changes were not related to age, PMI, or pH of the brain. Furthermore, these changes were not associated with mental disorders, as no statistically significant differences were observed either in PKC activity or in protein or mRNA expression of PKC α , β , and γ isozymes between suicide victims with no history of mental disorders and those with a history of mental disorders in either the PFC or the hippocampus. However, PKC activity and protein and mRNA expression of the PKC isozymes in suicide victims with or without mental disorders still were statistically significantly lower than in control subjects. This observation has provided preliminary evidence suggesting that alterations in PKC activity and isozymes in teenage suicide victims are independent of diagnosis. However, these results need to be replicated in a larger number of suicide victims with or without mental disorders before arriving at a definite conclusion on the effect of mental disorders on PKC isozymes.

There is direct and indirect evidence suggesting that PKC may play an important role in the pathogenesis of mood disorders. Indirect evidence is derived from the observations that treatment with lithium and other mood-stabilizing drugs causes a decrease in PKC activity and expression of certain PKC isozymes in rat brain.³² Because this effect of lithium is also shared by other mood-stabilizing drugs, such as valproate,³² this implies that a PKC abnormality may be associated with bipolar illness and that treatment with lithium or other mood-stabilizing drugs may normalize this abnormality. Direct evidence in support of the involvement of PKC in mood disorders is limited and is derived mainly from studies of PKC in platelets obtained from patients with mood disorders. For example, a previous study²³ reported that PKC activity is decreased in platelets of bipolar patients compared with control subjects. This decrease in PKC activity was mainly due to a selective decrease in the protein expression of PKC α , β I, β II, and δ in membrane and cytosol fractions of platelets from bipolar patients. Friedman et al²⁴ and Young et al³³ reported that PKC activity is increased in platelets of bipolar patients during the manic phase. Young et al³³ studied the expression of PKC α in these patients and did not find any difference in cytosol and membrane fractions of platelets obtained from bipolar patients.

Protein kinase C has not been studied extensively in the postmortem brain. In a recent study, Pandey et al²⁵ determined PKC binding sites using [³H]PDBu, which is a measure of the regulatory binding site of PKC, in the PFC of teenage suicide victims and nonpsychiatric control subjects and found that [³H]PDBu binding was significantly decreased in teenage suicide victims compared with control subjects. Coull et al³⁴ also studied [³H]PDBu binding in the PFC and hippocampus of antidepressant-treated and antidepressant-free adult depressed suicide victims. They did not find any significant differences in [³H]PDBu binding between antidepressant-treated suicide victims and control subjects. On the other hand, they found a significant increase in the B_{\max} of [³H]PDBu binding in the soluble fractions of antidepressant-free suicide victims compared with control subjects. The apparent differences

between our study and that of the depressed suicide victims reported by Coull et al³⁴ could be due to the difference in age of the populations studied. It is possible that the changes in PKC may be different in teenage vs adult suicide victims because of developmental factors and factors associated with chronicity of illness or previous long-term treatment with psychoactive drugs. Dean et al³⁵ reported a significant decrease in the density of PKC in the parahippocampal gyrus of patients with schizophrenia compared with control subjects. The results of studies of PKC in postmortem brain thus seem to be mixed; however, almost all studies in the postmortem brain of suicide victims, patients with schizophrenia, or depressed suicide victims were carried out using [³H]PDBu for labeling PKC binding sites, but none of these studies examined the protein or mRNA expression of individual PKC isozymes. Although [³H]PDBu has been used for studying PKC binding sites, it is believed that [³H]PDBu also labels other proteins, such as α -chimerins and RAS-GRP, and it is possible that the failure to find any changes in PKC binding sites may not reflect abnormal expression of PKC protein. Studies of PKC activity and of expression of the various PKC isozymes may, therefore, be important in examining the role of PKC in these disorders. To our knowledge, this is the first study to examine protein and mRNA expression and PKC activity in postmortem brains obtained from psychiatric patients and suicide victims. As mentioned earlier herein, we found that the expression of 3 different PKC isozymes—PKC α , β , and γ —was significantly decreased in the PFC and hippocampus of teenage suicide victims.

The mechanism by which PKC is decreased in the postmortem brain of suicide victims is not clear. The activation of PKC by an agonist such as diacylglycerol or phorbol myristate acetate causes the translocation of PKC from the cytosol to the membrane. This translocation also facilitates the proteolytic degradation of the enzyme. Another possibility could be related to a compensatory mechanism in response to the sustained activation of the receptors to which this signaling cascade is linked. For example, our group⁵ recently reported that 5-HT_{2A} receptors are increased in the postmortem brain of teenage suicide victims. It is possible that activation of these increased receptors may cause continued activation of the enzyme and the down-regulation of PKC and some of its specific isozymes. That this mechanism may be a reason for the decrease in PKC in the postmortem brain of suicide victims is supported by the observation of some investigators that continued activation of the enzyme PKC by phorbol esters causes its translocation and subsequent degradation and decrease.³⁶ The other reason for a possible decrease in PKC may be related to an overactive hypothalamic-pituitary-adrenal (HPA) axis in suicide. It has been reported that the HPA axis may be abnormal in suicide victims.³⁷ Earlier, our group³⁸ showed that activation of the HPA axis causes down-regulation of certain PKC isozymes in rat brain. Therefore, it is possible that the changes in PKC may be related to altered HPA function in suicide victims.

The pathophysiologic significance of the decrease in PKC in suicide victims remains to be elucidated;

however, the phosphorylation of proteins, mediated by PKC, is a key to many physiologic functions in the brain, including gene transcription. Some examples of proteins phosphorylated by PKC are MARCKS and GPA-43. Both these proteins have been implicated in mood disorders.^{23,32} Furthermore, certain evidence indicates that besides phosphorylating proteins in cytosol, PKC α , β , and γ translocate into the nucleus and phosphorylate many nuclear proteins,^{39,40} cell cycle-related proteins,⁴¹ and transcription factors, such as CREB and NF κ B.^{42,43} Although specific functions of each PKC isozyme are not clearly known, recent studies demonstrate that PKC α is closely associated with proliferation, cell differentiation, and metabolism and with some cell type-specific functions, such as β -cell development and activation.^{44,45} As far as PKC γ is concerned, it is the only PKC isozyme that is present solely in the brain and spinal cord.^{16,46} Many neuronal functions have been attributed to PKC γ , among them synaptic formation, long-term potentiation, long-term depression, and modulation of receptor functions, particularly γ -aminobutyric acid A.⁴⁷ Protein kinase C γ -deficient mice exhibit impaired motor coordination and deficits in spatial and contextual learning.⁴⁸ Mutation in PKC γ also shows brain pathology resembling the parkinsonian syndrome.^{49,50} Whether a deficiency in PKC is associated with suicidal behavior is not clearly known; however, given the many substrates that PKC phosphorylates, a deficiency in PKC isozymes may disrupt normal brain function.

Regardless of the mechanism by which PKC is down-regulated in the postmortem brain of suicide victims, our observation that PKC activity and some of its isozymes are decreased in the postmortem brain of teenage suicide victims may be vitally important in understanding the neurobiologic abnormalities of suicide.

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Correction

Errors in Table. In the Original Article by Korszun et al titled "Familiality of Symptom Dimensions in Depression," published in the May issue of the ARCHIVES (2004;61:468-474), errors occurred in Table 3 on page 472. In that table, the P values for fatigability and exhaustion, anxious foreboding with autonomic symptoms, suicidality, general rating of phobias, subjective feeling of retardation, and anhedonia should have been listed as <.001.